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Ion Channel Conductance Measurements on a Silicon-Based Platform

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Abstract. Conductance measurements of the transmembrane porin protein OmpF as a function of pH and bath concentration have been made with both a microfabricated silicon substrate device and a commercially available polystyrene aperture. Ion transport through the channel was simulated in atomic detail: the measured current was compared with theoretically calculated current, using a Brownian Dynamics kernel coupled to the Poisson equation by a P3M force field. The explicit protein structure and fixed charge distribution in the protein are calculated using the molecular dynamics code, GROMACS. Reasonable agreement is obtained in the simulated versus measured conductance over the range of experimental concentrations studied.

1. Introduction

Ion channel proteins provide low resistance paths that allow the diffusion and migration of ions and small molecules between intra- and extra-cellular regions separated by the otherwise high resistance lipid barrier of the cell membrane. These channels are of considerable biological interest because they are the control molecules of many of life's functions, much as transistors are the control devices of many computer functions. Single ion channel proteins are routinely measured in many biology laboratories using the patch-clamp technique which sucks a small area of cell into a pipette isolating proteins in that region [1] or the reconstitution methods of Montal and Mueller [2]. The formation of a giga-seal between the cell membrane and pipette tip limits leakage current and allows for the recording of ionic current through the transmembrane channels. Considerable efforts have been made to fabricate small apertures in glass [3], Si/SiO_2 [4, 5] and silicone elastomers [6] to planarize the patch-clamp. Microfabrication of parallel apertures could be used for high throughput screening methodologies in both pharmaceutical and integrated biological sensor applications.

Previously, we have demonstrated microfabricated silicon substrates as a universal platform suitable for recording the electrical activity of ion channel proteins inserted into suspended bilayer

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membranes [5, 7]. The bilayers span 150μm diameter apertures etched into silicon substrates using standard microelectronics processing techniques. Reversible Ag/AgCl electrodes are integrated around the circumference of the aperture and provide long-term stable measurements of the ion channel currents [7].

We have measured the conductance of OmpF porin ion channel proteins inserted into a lipid bilayer formed using Montal-Mueller methods [2]. OmpF is a transmembrane protein with a trimeric configuration composed of three β-barrel monomers with nanometer scale dimensions. Systematic measurements of the lipid giga-seal characteristics have been performed, including AC conductance measurements and statistical analysis in order to resolve the conductance of individual ion-channels. The measured conductance of OmpF porin has been compared to simulations with a Brownian Dynamics kernel self-consistently coupled to Poisson equations using a P3M force field scheme and the GROMACS description of protein structure and permanent charge.

2. Experimental

Samples were prepared using 4", double-sided polished Si (100) wafers having a thickness of 440μm. The substrates were patterned using photolithography and standard AZ4330 resist and then etched in a deep silicon reactive ion etcher (STS Advanced Silicon Etcher) using the Bosch process. The aperture was designed to have a 150μm diameter centrally located in a 1mm diameter region thinned to a final thickness of 150μm. A thermal oxidization of 200nm followed to produce an electrically insulating layer on the surface. The device was then coated with 75μm of SU-8 decreasing the overall capacitance of the device. Next, 8000Å of silver was evaporated onto the surface of both sides of the wafer with a CHA 600-SE electron beam evaporator. On the side of the device with exposed oxide, a 20nm adhesion layer of titanium was deposited prior to silver deposition. A PTFE layer was then chemically vapor deposited using the deep reactive ion etcher with C_4F_8 as the gas source. The hydrophobic termination lowers the surface energy and increases the contact with the lipid hydrocarbon chains, thereby allowing formation of a high resistance giga-seal. Finally, the electrodes were chloridized in 5% NaOCl resulting in AgCl electrodes integrated onto the surface of the device. All fabrication was performed at Arizona State University in the Center for Solid State Electronics Research cleanroom.

Lipid bilayer experiments were performed using a Teflon bilayer chamber with a 5 mm diameter opening between two baths of electrolyte solution. Both baths were filled with 3 ml KCl solution (1.0M for the bilayer measurements and 0.75M for the OmpF porin measurements), buffered with 20 mM N-(2-Hydroxyethyl) piperazine-N'- (2-ethanesulfonic acid) (HEPES) at pH 7.4. The device was sandwiched between the baths with the aperture in the center of the opening. Lipids (1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine and 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine) (DOPE: DOPC, 4:1) were dissolved in n-Decane (10mg/ml) and used to form a bilayer with the techniques of Montal and Mueller [2]. Current response and channel conductance were measured using an Axon Instruments Axopatch amplifier, a Stanford Research Systems SR 830 lock-in amplifier, a Stanford Research Systems SR570 current preamplifier and a National Instruments DAQ PCI card programmed with LabVIEW software. A commercially available polystyrene aperture from Warner Instruments was used for conductance measurements for comparison purposes.

3. Results and Discussion

Figure 1A shows the recorded conductance of OmpF porin for a 1M KCl solution, with a 100mV box-car serving as an input bias signal, and a 25 Hz sinusoidal AC signal superimposed on the box-car input. A histogram of the recording shows clustering of the measured conductance occurs into peaks separated by nearly constant conductance intervals (Figure 1B). The first peak corresponds to the conductance of the bilayer itself with no protein inserted, which, as alluded to earlier, is on the order of giga-ohms in terms of its resistance. Subsequent peaks correspond to the conductance of one conducting channel, then two, and so forth.

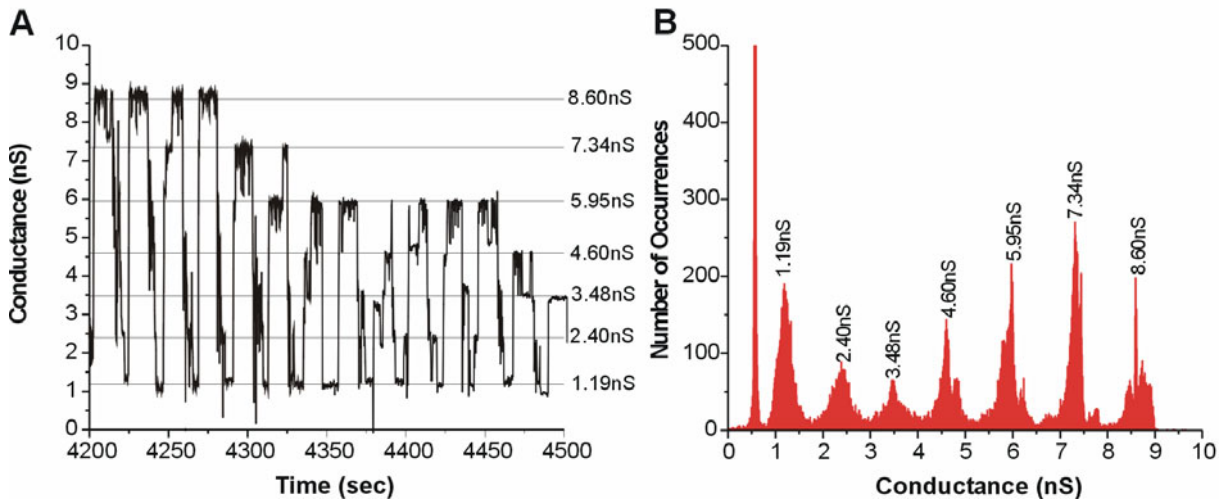

Figure 1. OmpF porin was inserted into lipid bilayers suspended on the commercial device and measured using phase sensitive techniques in 1.0M, pH 7.4 KCL solution (A). A statistical histogram of the complete data set shown partially in (A) was compiled where each peak corresponds to single pore of OmpF (B).

The average measured conductance per channel for a given KCl concentration is then determined as the average of the difference between successive peaks, which is shown in Figure 2 (pH 7.4 circles and pH 8.0 triangles). These measurements are a compilation of both phase sensitive lock-in techniques and voltage clamp techniques on the microfabricated and commercial apertures [7]. Here we observe a decreasing conductance with decreasing KCl concentration, as expected. There is an apparent offset in the conductance for zero concentration, which is somewhat unexpected, probably representing current through a different part of the OmpF protein, that does not open and close. The sensitivity of the offset to the pH and buffering system are currently under investigation.

Brownian dynamics methods are attractive for modeling conduction in porin because they allow for simulation times that can provide statistically relevant information about the ion permeation process. The ion trajectories are calculated by the stochastic solution of the full Langevin equation, integrated with a third order scheme and a free flight time step of 20fs. In the simulation code the BD kernel is coupled to a real space Poisson P3M force-field scheme [8-10] to account for the electrostatic interactions, including the external boundary conditions and dielectric interfaces. A third order triangular shaped cloud (TSC) scheme [8] is used for the charge assignment and force interpolation and the van der Waals interactions are included using the standard Lennard-Jones potential. The time step used to update the solution of Poisson's equation during the simulation is 2ps.

In the computational domain the lipid membrane is represented as a fixed dielectric slab 3nm wide. The ion channel is embedded in the membrane and is represented as a rigid structure. The dielectric constant of the membrane and channel is 2.0 and 4.0, respectively. The water in the surrounding electrolyte bath is also treated as a continuum and assigned a dielectric constant of 80. The charge distribution of the protein atoms is determined with the software package GROMACS [11], starting from the atomic coordinates of the OmpF structure as determined by X-ray diffraction [12]. The protonation states of the protein residues are taken at neutral pH, resulting in a net charge of -30e for the entire porin trimer. The channel/membrane system is mapped onto a 30×30×50 inhomogeneous Cartesian grid with a mesh spacing that varies from 1nm in the bulk electrolyte solution to 0.2nm in the interior of the channel, maintaining a mesh expansion ratio less than 1.5 the grid. Dirichlet conditions are used at boundaries perpendicular to the permeation pathway of the porin to include the externally applied bias. An artificially high voltage of 1.0V is used to improve the signal-to-noise ratio. Since the channel/membrane system is treated as a rigid insulating structure, the high voltage will not impact the simulated measurements of the conductivity. Results of the modeling of the OmpF

porin concentration are shown in Figure 2 (squares). The lower modeled conductance might be increased by changing the protein dielectric constant, examining the impact of different protonation states of the protein residues, and the inclusion of a flexible protein structure.

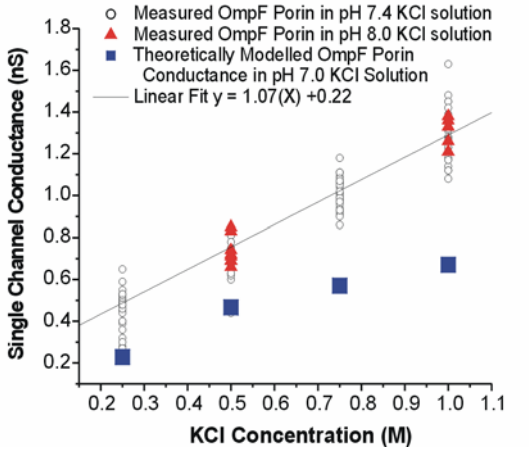

Figure 2. Measurements of the conductance of OmpF porin as a function of KCl bath concentration using both voltage clamp techniques and AC lock-in amplifier techniques at a pH of 7.4 (open circles) and pH 8.0 (triangles). A linear fit of the average conductance at pH 7.4 is shown with the measured data. Data points from a theoretical simulation modelling the conductance of the OmpF porin at different KCl concentration were slightly lower then measured values (squares).

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